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T. D. Horne  
PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of  
Catherine HANNI et al.  
Serial No. 09/423,259  
Filed March 2, 2000



GROUP 1655

Examiner J. Eismann

METHOD FOR DETECTING THE PRESENCE  
OF BIOLOGICAL MATTERS OF BOVINE  
ORIGIN, AND OLIGONUCLEOTIDES FOR  
ITS IMPLEMENTATION

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AMENDMENT

TECH CENTER 1600/2900

Commissioner for Patents

Washington, D.C. 20231

Sir:

Responsive to the Official Action of November 6, 2000, please amend the above-identified application as follows:

IN THE CLAIMS:

Cancel claims 1-16 without prejudice, and add the following new claims:

--17. (new) Oligonucleotides consisting of (a) about 15 to 25 nucleotides contained in SEQ ID NO: 1 or (b) SEQ ID NO: 2 or (c) SEQ ID NO: 3 wherein Y is T or C.

--18. (new) Oligonucleotides consisting of (a) about 15 to 25 nucleotides contained in SEQ ID NO: 4 or (b) SEQ ID NO: 5 or (c) SEQ ID NO: 6.

--19. (new) Pairs of primers consisting of one of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3; and one SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6.

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--20. (new) Pairs of primers according to claim 19, wherein said pairs of primers consist of the oligonucleotides of SEQ ID NO: 3 and SEQ ID NO: 6.

--21. (new) Pairs of oligonucleotide primers, each primer being selected from the group consisting of (a) a sequence of about 15 to 25 nucleotides of SEQ ID NO: 9; (b) SEQ ID NO: 10; (c) SEQ ID NO: 11; (d) SEQ ID NO: 12; (e) SEQ ID NO: 13; and (f) SEQ ID NO: 14.

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*CONT'D*

--22. (new) Pairs of oligonucleotide primers according to claim 21, wherein said pairs of primers are selected from the group of primer pairs consisting of: SEQ ID NO: 9 with SEQ ID NO: 10; SEQ ID NO: 6 with SEQ ID NO: 11; SEQ ID NO: 12 with SEQ ID NO: 3; and SEQ ID NO: 13 with SEQ ID NO: 14.

--23. (new) Oligonucleotide consisting of at least 15 and up to all nucleotides of SEQ ID NO: 7 or SEQ ID NO: 19.

--24. (new) Probes, characterized in that they comprise oligonucleotides according to claim 23 or complementary or inverse/complementary oligonucleotides to the oligonucleotides according to claim 23.

--25. (new) DNA fragment consisting of SEQ ID NO: 8.

--26. (new) DNA fragments consisting of a sequence of at least 15 nucleotides contained in one of SEQ ID NOS: 15, 16, 17 and 18.

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CONCLD*

--27. DNA fragments according to claim 26, selected from the group consisting of: all 159 base pairs of SEQ ID NO: 15; all 153 base pairs of SEQ ID NO: 16; all 265 base pairs of SEQ ID NO: 17; and all 158 base pairs of SEQ ID NO: 18.--

**R E M A R K S**

All of the claims previously pending in this application are cancelled herewith, and new claims 17-27 are introduced. The new claims 17-27 present the elected subject matter in a manner which is believed to be proper as to form, adequately supported by an enabling disclosure, and clearly patentable relative to the prior art of record.

The rejections applied in the outstanding Official Action are of course obviated by the cancellation of all of the claims that were subject to those rejections. For the sake of expediting prosecution of the new claims to allowance, those rejections will be briefly addressed below, noting wherein they do not apply to the new claims 17-27.

At Item 4 of the Official Action, claims 4, 5, 7-9, 14 and 15 were rejected under the first paragraph of 35 USC §112, as allegedly being based on a non-enabling disclosure.

The rejection was based on the recitation of various percentages of sequence identity in the previous claims.

Those recitations do not appear in any of new claims 17-27, and it is therefore believed to be apparent that the non-enablement rejection should not be applied to any of the new claims.

At Item 6, previous claims 4-9 and 13-15 were rejected for indefiniteness.

The various informalities noted by the Examiner with respect to the previous claims do not appear in any of the new claims. Consequently, it is believed to be evident that the indefiniteness rejection applied against the previous claims should not be applied against any of the new claims.

At Item 8 of the Official Action, previous claims 4, 5, 8, 9 and 13-15 were rejected as allegedly being anticipated by LOFTUS et al. ("Evidence for two independent domestications of cattle", Proc. Natl. Acad. Sci. USA, Vol. 91, March 1994, pages 2757-2761). In addition, at Item 10 of the Official Action, claims 6 and 7 were rejected as allegedly being unpatentable based on the combined disclosures of LOFTUS et al. in view of FEI et al. (Animal Science and Technology, Vol. 67, No. 10, 1996, pages 900-905). Both of those grounds of rejection are respectfully traversed, for the following reasons.

By way of brief review, the present invention concerns a method for obtaining a bovine DNA fragment having predetermined size and sequence, particular to bovines, the method comprising amplifying, by chain polymerization reaction (PCR reaction), a predetermined bovine genome sequence present

in bovine genomes. More particularly, the invention relates to specific mitochondrial DNA fragments of the bovine genome having a sequence identical to the extent of at least 80% to homologous regions of the sequence of the control region of the mitochondrial DNA.

1) Concerning the novelty of the present invention, the applicants deem that, even if the teaching of LOFTUS et al. comprises all of the instantly claimed sequences, the claimed sequences (that is to say, the oligonucleotides, the probes and the fragments) are nevertheless novel, because the claimed sequences are in fact fragments (that is to say "parts") of the sequences disclosed in LOFTUS et al. (more particularly in GenBank Accession number L27725 or L27712), and these fragments (or "parts") according to the present invention are not specifically described in the state of the art. Thus, even if the claimed sequences are comprised in the sequences disclosed by LOFTUS et al., the claimed sequences are novel because they have never been isolated or selected as such.

2) Concerning the non-obviousness of the present invention, the applicants deem that it was not obvious to select pairs of oligonucleotide primers, as well as oligonucleotides, probes and fragments as claimed, inside the disclosed sequences, for the reasons indicated hereafter.

(1) First, the oligonucleotides primers according to the present invention have to be specific to the bovines (that is to say, to the *Bos taunus* species) and should not amplify

other species such as goat or sheep. Many sequences having about 20 nucleotides and chosen in the disclosed sequence (GenBank Accession number L27725 or L27712) could not be used for the purpose of the present invention (that is to say, detecting the presence of biological matters of bovine origin), because the control regions of the mitochondrial DNA (mtDNA) (D-loop) of the bovines present a certain percentage of sequence identity with goat or sheep.

Moreover, the oligonucleotide primers according to the present invention have to amplify all of the bovine breed, whatever their geographic origin.

This double requirement makes the determination of oligonucleotides relatively complex.

(2) The second point is that the oligonucleotide primers according to the present invention have to allow the amplification of a DNA fragment relatively short (maximum 250-300 bp) in order to be amplified in degraded substrates (this point represents a very important application in the present invention).

Moreover, the amplified sequences have to contain, despite their low number of base pairs, enough information in order to allow the identification between the species which are to be distinguished. By way of example, this means that the amplified sequences allow the identification between two species, if they contain more than about 1% of different nucleotides. As an example, it will be possible to distinguish two species from each other if the two amplified

sequences (having about 200 base pairs) contain more than 5 different nucleotides.

This double requirement makes the determination of appropriate oligonucleotides relatively difficult.

Another point is that the two oligonucleotide primers which are used together have to be compatible, that is to say, have to hybridize with the target DNA in optimal temperature and salinity conditions.

(3) The applicants draw the Examiner's attention to the fact that some bovine mitochondrial DNA regions disclosed in the state of the art (LOFTUS et al. or FEI et al.) could not be appropriate to amplify bovine breeds, and thus used for detecting the presence of biological matters of bovine origin.

a) The sequences called respectively A, B, C, D, E, F, G, H, I and J in the examples given below, and disclosed in LOFTUS et al., demonstrate that some bovine mitochondrial DNA regions could not be appropriate to develop primers according to the present invention.

The nucleotide positions indicated in the examples below "according to ANDERSON et al.", were determined according to the complete mitochondrial DNA sequence of beef, which comprises 16 338 nucleotides, determined by ANDERSON et al., 1982, J. Mol. Biol., 156, 683-717.

Moreover, the applicants indicate the nucleotide positions of the sequence disclosed in GenBank Accession number L27725 (LOFTUS et al.).

- The sequence A: 5'-CGTGAAACCAGCAACCCGCT-3' (nucleotides 16,175 to 16,194 according to ANDERSON et al., or nucleotides 371 to 390 of the sequence disclosed in GenBank Accession number L27725) is highly conserved between beef, sheep, goat, pig and even, to a lesser extent, in humans.

- The sequence B: 5'-ATCGAGATGTCTTATTAAAG-3' (nucleotides 16,317 to 16,336 according to ANDERSON et al., or nucleotides 513 to 532 (see the complimentary strand) of the sequence disclosed in GenBank Accession number L27725) is perfectly conserved (100% identity) between all the regarded species (human comprised).

Thus, the use of the pair of primers AB could not allow to specifically detect beef, because a sample comprising sheep, goat or pig would also be amplified.

The sequences A and B are comprised of GenBank Accession number L27725 (LOFTUS et al.) but cannot be used for the purpose of the present invention (method for detecting the presence of biological matters of bovine origin).

The same is true for the following sequences:

- Sequence C: CTAATGGCTAATCAGCCCAT (nucleotides 2 to 21 according to ANDERSON et al., or nucleotides 536 to 555 of the sequence disclosed in GenBank Accession number L27725),
- Sequence D: TTTGACGGCCATAGCTGAGT (nucleotides 81 to 100 according to ANDERSON et al., or nucleotides 615 to 634 (see the complementary strand) of the sequence disclosed in GenBank Accession number L27725),

- Sequence E: CCCTTAAATATCTACCACCA (nucleotides 237 to 256 according to ANDERSON et al., or nucleotides 772 to 788 of the sequence disclosed in GenBank Accession number L27725),
- Sequence F: AATATGTATATAGTACATT (nucleotides 15,994 to 16,013 according to ANDERSON et al., or nucleotides 190 to 208 of the sequence disclosed in GenBank Accession number L27725),
- Sequence G: ATTTGACATAATGTACTATG (nucleotides 16,089 to 16,109 according to ANDERSON et al., or nucleotides 285 to 304 (see the complementary strand) of the sequence disclosed in GenBank Accession number L27725),
- Sequence H: CACCACTAGCTAACATAACA (nucleotides 15,900 to 15,919 according to ANDERSON et al., or nucleotides 96 to 115 of the sequence disclosed in GenBank Accession number L27725),

Moreover, some other sequences are not appropriate because they are too simple (in particular because they contain a lot of identical repeated nucleotides), and do not allow a correct amplification. This is the case of the sequences I and J described below.

- Thus, Sequence I: 5' -TTTTTTTATTTGGGGGATG (nucleotides 56 to 75 according to ANDERSON et al., or nucleotides 590 to 609 of the sequence disclosed in GenBank Accession number L27725), and
- Sequence J: 5' -AACGGGGGGGGGGGGCCTG (nucleotides 348 to 366 according to ANDERSON et al., or nucleotides 883 to 893 (see the complementary strand) of the sequence disclosed

in GenBank Accession number L27725), could not give correct results in PCR reaction.

Moreover, the applicants draw the Examiner's attention to the fact that the sequence J disclosed in FEI et al. is very close (see in particular the six last nucleotides at the 5' end of the sequence J "5'-AACGGG") to the primer called "BR" in FEI et al. (see in particular the six last nucleotides at the 3' end of the primer BR for cattle "AACGGG-3'"). The primer BR disclosed in FEI et al. being very close to a simple region (that is to say, containing one base very repeated, such as 12 G) will not be efficient on degraded DNA.

b) The examples given below also demonstrate that it is difficult to determine appropriate oligonucleotides, which have only to be specific towards bovines.

The line 1 of the following example represents the nucleotides 254 to 288 of the bovine control region of the mitochondrial DNA determined by ANDERSON et al., 1982, J. Mol. Biol., 156, 683-717.

Lines 2,3 and 4 respectively represents:

- nucleotides 396 to 430 of the sheep (*Ovis aries*) control region of the mitochondrial DNA, disclosed in Seq genbank AF039577, positions 396-450 (sequence coming from "Hiendleder S, Mainz K, Plante Y, Lewalski H. Analysis of mitochondrial DNA indicates that domestic sheep are derived from two different ancestral maternal sources: no evidence for contributions from urial and argali sheep. J. Hered. 1998 Mar-Apr; 89(2):113-20"),

• nucleotides 405 to 439 of the goat (*Capra hircus*) control region of the mitochondrial DNA, disclosed in Seq genbank AB004076, positions 405-439, (sequence coming from "Takada T, Kikkawa Y, Yonekawa H, Kawakami S, Amano T. Bezoar (*Capra aegagrus*) is a matriarchal candidate for ancestor of domestic goat (*Capra hircus*): evidence from the mitochondrial DNA diversity. *Biochem Genet.* 1997 Oct; 35(9-10):315-26."),

• nucleotides 399 to 43 of the pig (*Sus scrofa*) control region of the mitochondrial DNA, disclosed in Seq genbank AB015092 positions 399-433, sequence coming from "Okumura N, Ishiguro N, Nakano M, Hirai K, Matsui A, Sahara M. Geographic population structure and sequence divergence in the mitochondrial DNA control region of the Japanese wild boar (*Sus scrofa leucomystax*), with reference to those of domestic pigs. *Biochem Genet.* 1996 June; 34(5-6):179-89).

The oligonucleotides 1, 2 and 3 were made by the inventors as examples in order to demonstrate that it is difficult to determine appropriate oligonucleotides, which have only to be specific towards bovines.

Beef	5' -CCACTTTAACAGACTTTCCCTAGATACTTATTT-3'
Sheep	...T.....CG...CC.....T.A..A.
Goat	..GT...A....CG..CCCT.....T.AG.GC
Pig	..CA.....A.CTTA.GC.T...C.C..CC..A.
Oligo 1	5' -CCACTTTAACAGACTTT-3'
Oligo 2	5' -AACAGACTTTCCCTAGATA-3'
Oligo 3	5' -AGACTTTCCCTAGATACTT-3'

The point means that it concerns a sequence identity with the corresponding beef position.

The inventors of the present invention have determined that the 3' part of an oligonucleotide has to present a sequence region which is specific, in order to obtain a specific detection of the chosen species (in the present case the beef). The rest of the sequence have to be "relative" identical (no more than 4 or 5 different bases for an oligonucleotide having 20 base pairs).

In the above example, the oligonucleotide 1 is specific towards bovines because the 3' part is different between the beef and the three other species (sheep, goat and pig). Oligonucleotide 2 is not specific towards beef, because it will recognize sheep and goat, and in a less extent, pig. Oligonucleotide 1 is better than oligonucleotide 3, which is better than oligonucleotide 2.

More precisely, the inventors have determined that an oligonucleotide is ideal when its three last 3' bases (in the present case TTT for oligonucleotide 1) are very specific towards the searched target. Thus, oligonucleotide 2, which two of the three last bases 3' are similar to the pig (ATA against ACA) will not be good specific. Oligonucleotide 3 will distinguish bovine from pig as well as oligonucleotide 1 will distinguish bovine from goat, however, oligonucleotide 3 will be less efficient to distinguish bovine from sheep or goat, because even if the last base is different (T in oligonucleotide 3 and A in sheep or goat), the next to last

base is the same in the three cases (T in oligonucleotide 3, in sheep and in goat).

Conclusion:

The above examples (see points a) and b)) demonstrate that the sequences disclosed in LOFTUS et al. or FEI et al. are not sufficient to elaborate specific oligonucleotides according to the present invention. In fact, a lot of different oligonucleotides can be prepared in a sequence containing 1000 base pairs, and since there oligonucleotides are used in pairs, the total number of possibilities is very large.

c) Another very important point lies in the fact that it is expressly indicated in the LOFTUS et al. publication that "mtDNA was isolated from fresh blood samples..." and that "Primers, derived from the unknown bovine mtDNA sequence were designed to lie in the conserved proline tRNA and 12S rRNA genes." (see page 2757, column 2, last paragraph of the above-mentioned publication).

Thus, the oligonucleotides used by LOFTUS et al. for generating the sequences L27725 and L27712, are not in the control region of the mtDNA (but in two adjacent genes) and these oligonucleotides were chosen because they are preserved in a vast range of vertebrate, and this choice is precisely what the inventors of the present invention wanted to avoid.

(4) The applicants also draw the Examiner's attention to the fact that, even if sequences are disclosed in published bank sequences, it is possible to obtain patents for fragments or parts of the disclosed sequences, when the said

fragments or parts have never been isolated or selected as such, and when these fragments or parts are used in a definite purpose.

As an example, U.S. Patent No. 5,596,089 relates to novel bovine and porcine genomic sequences for the SRY gene, a method for genetic sex determination of bovine or porcine tissue, and a method for the genetic selection of sexual phenotype in domestic animals. Claim 1 of the said patent concerns an oligonucleotide probe which comprises the nucleotide sequences selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 1 and SEQ ID NO: 2 being relatively bovine and porcine genomic sequences for the SRY gene.

However, when this patent application was filed, the sequence of the SRY gene was published in bank sequences for a long time.

From the above discussion, therefore, it is believed to be apparent that neither the anticipation nor the obviousness rejections applied in the outstanding Official Action, could appropriately be applied to any of new claims 17-27.

In view of the present amendment and the foregoing remarks, therefore, it is believed that this application has been placed in condition for allowance, with new claims 17-27.

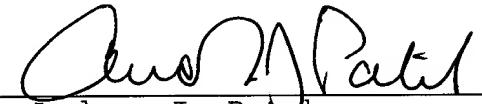
HANNI et al. S.N. 09/423,259

Allowance and passage to issue on that basis are accordingly respectfully requested.

Respectfully submitted,

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BY



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